Interactions between Chlorinated Dioxins and a Positively Charged Molecular Probe: New Molecular Interaction Potential

JAMES R. RABINOWITZ, STEPHEN B. LITTLE, ERIC M. GIFFORD*

Environmental Carcinogenesis Division (MD-68), National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Received 26 August 1997; accepted 2 December 1997

ABSTRACT: Interaction with the ligand binding domain of receptors for natural chemicals present one potential mechanism for the biological effects of environmental chemicals. Evidence suggests that the electrostatic interaction between the ligand and the receptor is an important component for binding to some of the relevant receptors. The presence of charged residues near the binding site suggests that the charge distribution of the free ligand may be different from the charge distribution of the ligand as it approaches the binding domain of the protein. In this study a new type of potential is computed for a series of dibenzo-*p*-dioxin (dioxin) ligands. This quantum mechanically computed potential results from interaction between the ligand and a trimethyl ammonium probe at a set of grid points. This interaction potential is compared with the molecular electrostatic potential computed from the wave function of the isolated ligands. Three types of local minima are found: (1) above the oxygen; (2) above the conjugated ring; and (3) above the chlorine(s). The molecular electrostatic potential emphasizes the minima associated with the chlorine atoms and, in that potential, the minima associated with the oxygen atoms disappear with chlorination. In the new potential, the minima over the oxygen atoms are maintained even in tetrachlorodioxin. As chlorination is increased the differences between the two potentials increases. The new potential shows the influence of the π-cation interaction, which is largest when there is little substitution on the ring. The presence of the probe induces a dipole

*Postdoctoral fellow in the program in Toxicology of the University of North Carolina

Correspondence to: J. R. Rabinowitz; e-mail: rabinowitz.james@epamail.epa.gov

Contract grant sponsor: EPA/UNC Toxicology Research Program

CCC 0192-8651 / 98 / 060673-12

component of 1 debye perpendicular to the plane of the ligand. Local minima in the interaction potential are then used as starting structures for the determination of the most stable ligand−probe complexes. The most stable structures are obtained from the minima associated with the oxygen atoms. These structures are stabilized by a hydrogen bond formation between the probe and the oxygen and the molecule is bent by 30° about the O—O axis. For this series of molecules, the new potential retains some of the features that determine the hydrogen bond whereas the molecular electrostatic potential does not. © 1998 John Wiley & Sons, Inc. J Comput Chem 19: 673−684, 1998

Keywords: molecular interaction potential; molecular electrostatic potential; dibenzo-*p*-dioxin; induced molecular dipole moments; π-charge interaction

Introduction

The capacity of chemicals introduced into the environment by human activity to interfere with natural biological processes has been demonstrated in a number of different circumstances. Interaction with the ligand binding domain of receptors for natural chemicals that mediate biological activity presents one potential mechanism for this interference. By this means, chemicals may initiate the cascade that leads to adverse health and environmental effects. Some specific examples are chlorinated dioxins binding to the ligand binding domain (LBD) of the cytosolic Ah receptor and some pesticides and other industrial chemicals binding to the LBD of steroid hormone receptors. Although amino acid sequence data are available for the LBD of many of the environmentally relevant receptors, the three-dimensional structure is currently not available for many of these receptors. However, information on the three-dimensional structure of the LBD of other, similar receptors is available.

Recently, crystal structures have been obtained for one of the retinoid receptors and a different retinoid receptor with bound ligand.[1,2] These receptors are from the superfamily of nuclear receptors that includes the steroid hormone receptors. From these results it has been determined that the electrostatic interaction between the ligand and the receptor is the most important component in binding and that there are positively charged residues near the binding site. These investigators described a binding motif in which the interaction with the ligand induces large changes in the protein structure.[3] Additionally, statistically based structure−activity studies,[4] using data obtained for the binding of pesticides and other industrial chemicals to an estrogen receptor preparation,[5] have shown that the electrostatic potential of the putative ligand is the most important feature for rationalizing the binding affinity. Whereas these arguments indicate that the electrostatic interaction between the ligand and the ligand binding site of the receptor is important and differential, the presence of a charged residue near the binding site suggests that the charge distribution of the free ligand may be different from the charge distribution of the ligand as it approaches the LBD of the protein.

The dioxins and many of the environmentally important chemicals that disrupt the endocrine system have conjugated regions. Studies have emphasized the importance of the π-system−cation interactions for stabilizing protein structures[6] and binding small cations.[7,8] This interaction is likely to play a role in the binding of environmentally relevant chemicals to the LBD of receptors. However, for many of the environmentally relevant receptors, the three-dimensional structure of the LBD has not been determined.

As a way of comparing the capacity of chemicals in this class to bind to the LBD of receptors that contain positively charged regions, we have computed a new type of potential, using a trimethylammonium cation (triMA) as a probe. The interaction of this probe with a series of dioxin ligands is computed at a set of grid points in a plane parallel to the plane of the dioxin molecule (see Fig. 1). The computed probe interaction potential (PIP) maps appear similar to the molecular electrostatic potential (MEP), but are the result of quantum mechanical calculations at each grid point. As a result, it incorporates higher order effects including the interaction between the probe and the change in the electronic distribution of the ligand. Because dioxins are planar molecules, a natural way to compute and study these potential functions is in a plane parallel to the molecular plane. We have chosen to use a trimethylammonium

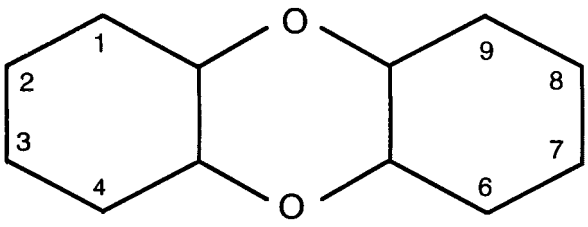

FIGURE 1. Dibenzo-*p*-dioxin (dioxin). Numbering of atoms as shown.

cation as a probe because it is a nitrogen-centered cation like the positively charged amino acids, but the computation is greatly simplified due to its small size. By choosing a probe with only a single N—H bond the complex problem of the orientation of the probe relative to the plane of the dioxin molecule is greatly simplified.[9]

Method

Potential functions for dioxin and six chlorinated dioxins were computed using a trimethylammonium probe. The nitrogen center of the probe was translated in 0.5-Å steps in a rectangular grid parallel to and 3.5 Å above the plane of the dioxin. The N—H bond of the probe was always perpendicular to the plane of the dioxin. As a result the H(N) was always 2.48 Å above the plane. The grid extended 3 Å in both directions beyond the Cl atoms in 2,3,7,8-tetrachlorodioxin, the largest molecule in the study, and was the same for each molecule. The structures of the dioxins and the probe were optimized separately. During the computation of the potential these structures were used unchanged. The potentials were computed at the Hartree–Fock level using the 3-21g* basis set and the Gaussian series of programs.[10] This basis set has *d*-functions only on the chlorine atoms. For some points on the grid, the counterpoise corrections were computed. When the probe was above the central part of the dioxin molecule the counterpoise correction was less than 2 kcal/mol, over the chlorines it was about 1 kcal/mol, and in the far corners of the grid it was near 0 kcal/mol.

For each local minima in the potential maps just described, and for a few other points, the structures of the dioxin and the probe were optimized together at the Hartree–Fock (HF) level. In this way, three-dimensional structures of the dioxin molecules under the influence of the probe were obtained. Whereas the probe orientation and position relative to the dioxin was changed by these computations its internal structure remained essentially unchanged.

Molecular electrostatic potentials were calculated for the dioxins using the methods available in Gaussian. In addition, potential derived charges, using the CHELPG[11] method, were obtained for the dioxin molecules and the probe and were used in an AMBER[12] force field to obtain a similar electrostatic interaction potential map. Multipole moments and atomic charges for the dioxins, under the influence of the probe, were computed. The Mulliken definition of atomic charge was applied to divide the charge distribution of the composite system between molecules (and atoms). Software developed in this laboratory was used to compute segmental multipole moments.[13] For comparison the dipole moments of the dioxins were computed in the presence of a perpendicular electric field using the PS-GVB program.[14]

Results

Figures 2–8 show the PIP and MEP generated for dioxin and six of the chlorinated dioxins in a plane parallel to the plane of the dioxin molecule. Both the PIP and the MEP for the dioxins show three types of local minima: (1) above the oxygen; (2) above the conjugated ring; and (3) above the chlorine(s).

For (nonchlorinated) dioxin the PIP contains a broad minimum over the central area of the molecule with the stable positions for the probe above the oxygen atoms and above the center of the conjugated rings. These positions have almost identical energies. For the MEP of the same molecule, the most stable minima are over the conjugated ring with less stable minima over the oxygen atoms. For both of these potentials there is a small maximum over the center of the molecule. The 3-kcal/mol difference between the maximum stabilization of the PIP and MEP is probably an underestimation of the effect of nonelectrostatic terms in the PIP because the center of charge of the triMA ion is farther from the plane of the dioxin molecule than 3.0 Å, the plane in which the MEP is calculated. The differences between the shape of the two types of potentials away from the minima is subtle. The PIP is attractive over a longer range than the MEP and both are quadrupolar at large distances.

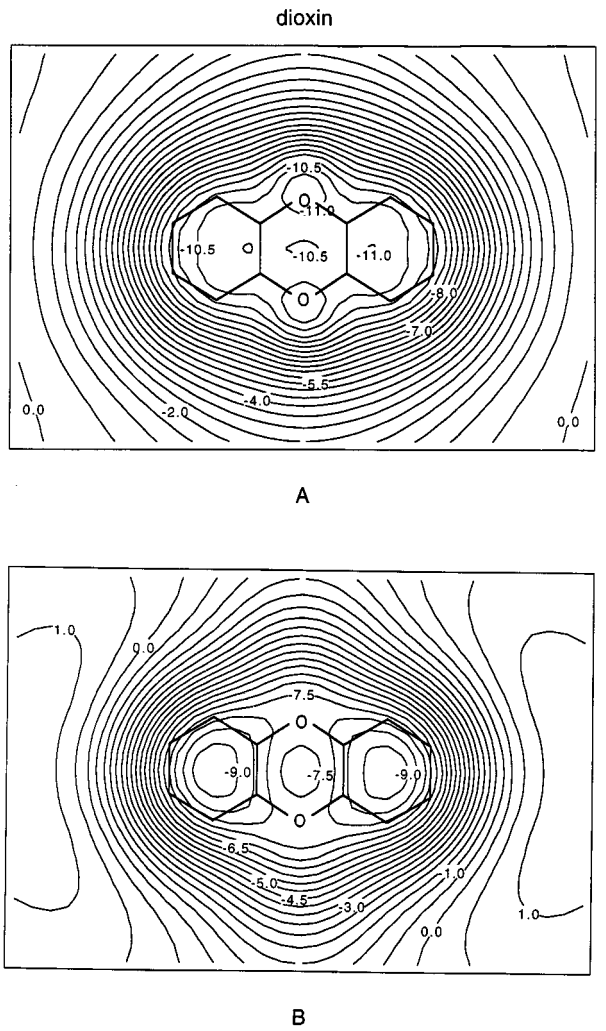

FIGURE 2. (A) The probe interaction potential (PIP) for unsubstituted dioxin, in a plane parallel to and 3.5 Å above the dioxin molecule. The probe interaction potential is defined as the difference between the energy of the complex when the nitrogen atom of the probe is at the point indicated and the energy of the dioxin and the probe when they are infinitely separated. (B) The molecular electrostatic potential of dioxin in a plane 3.0 Å above the molecule.

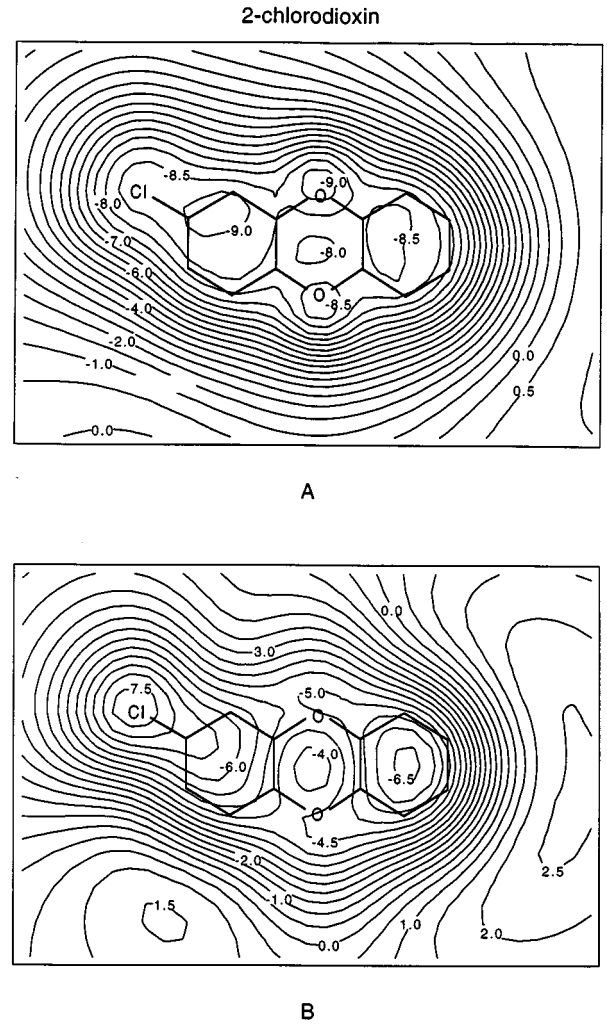

FIGURE 3. Same as Figure 2 except there is a chlorine atom substituted in the 2 position of the dioxin.

When a chlorine atom is added to the dioxin, the magnitude of the interaction described by the PIP decreases. The three types of minima just described are now present in both of these potentials. In the PIP the minima over the chlorine and the chlorinated ring have merged and the magnitude of that minimum is similar to the magnitude of the minima over the nonchlorinated ring and the oxygen atoms. The minima are (1–2) kcal/mol less stable than the similar minima in the nonchlorinated molecule. In the MEP there is a clear ordering of the three types of minima. The most stable minimum is associated with the chlorine and the minimum over the nonchlorinated ring is more stable than the minima over the oxygen atoms. The local minimum over the middle of the chlorinated ring is only seen in the perturbation it causes in the structure of the minimum over the chlorine atom at the current resolution. The difference between the PIP and the MEP is largest over the oxygens and the central nonconjugated ring and just outside the lateral hydrogen atoms, and smallest near the chlorine.

The magnitude of the local minima over the oxygen atoms in the PIP decreases as chlorine atoms are added to the dioxin structure, whereas in the MEP the corresponding local minima disappear completely. (The exception is 2,8-dichlorodi-

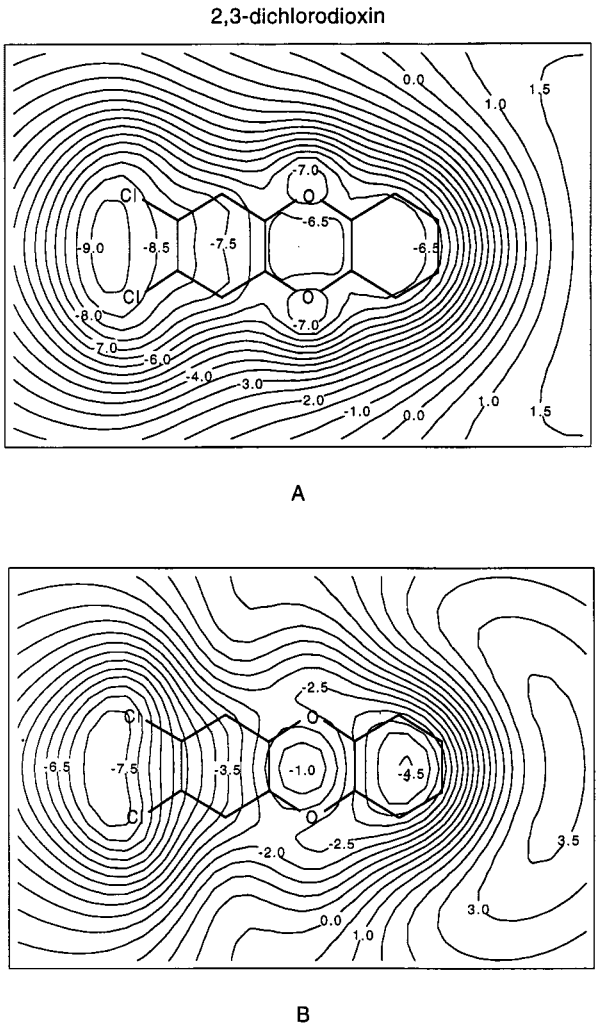

FIGURE 4. Same as Figure 2 except there are chlorine atoms substituted in the 2 and 3 positions of the dioxin.

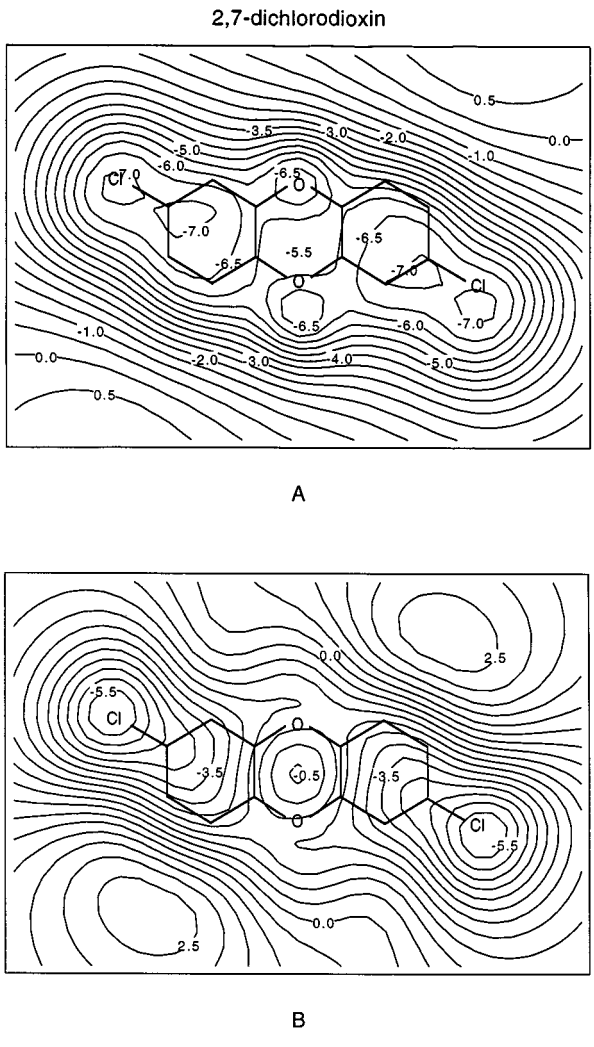

FIGURE 5. Same as Figure 2 except there are chlorine atoms substituted in the 2 and 7 positions of the dioxin.

oxin where the both chlorine atoms are along the same long axis of the molecule.) For the molecules in this set with three or four chlorine atoms, the region of the MEP over each oxygen atom is positive (repulsive). The minimum over the central region of the chlorinated ring disappears in both the PIP and the MEP for a conjugated ring with two chlorine atoms. For 2,3-dichlorodioxin (both chlorines on the same ring), the minimum over the central region of the nonchlorinated ring persists for both the PIP and the MEP, but is not as stable as the other minima. The most stable configuration for both the PIP and MEP is when the probe is above a chlorine atom for conjugated rings with a single chlorine substitution, or between the chlorine atoms when two chlorine atoms are on the same side of the system. As chlorine atoms are added to the molecule, the magnitude of all other local minima decrease.

As expected, the PIP is always more attractive than the MEP. However, the maximum difference in the magnitudes of the PIP and the MEP is 3.5 kcal/mol for the nonchlorinated molecule and 4.1 kcal/mol for the monochlorinated dioxin. It increases with the addition of chlorine atoms to the dioxin backbone and is 6.7 kcal/mol for tetrachloro. The smallest difference between the two potentials is found above the chlorine atoms whereas the largest difference is in the region of the oxygen atoms and central nonconjugated ring. There is also a maximum in the magnitude of the difference between the two potentials when the probe is just beyond the peripheral hydrogen

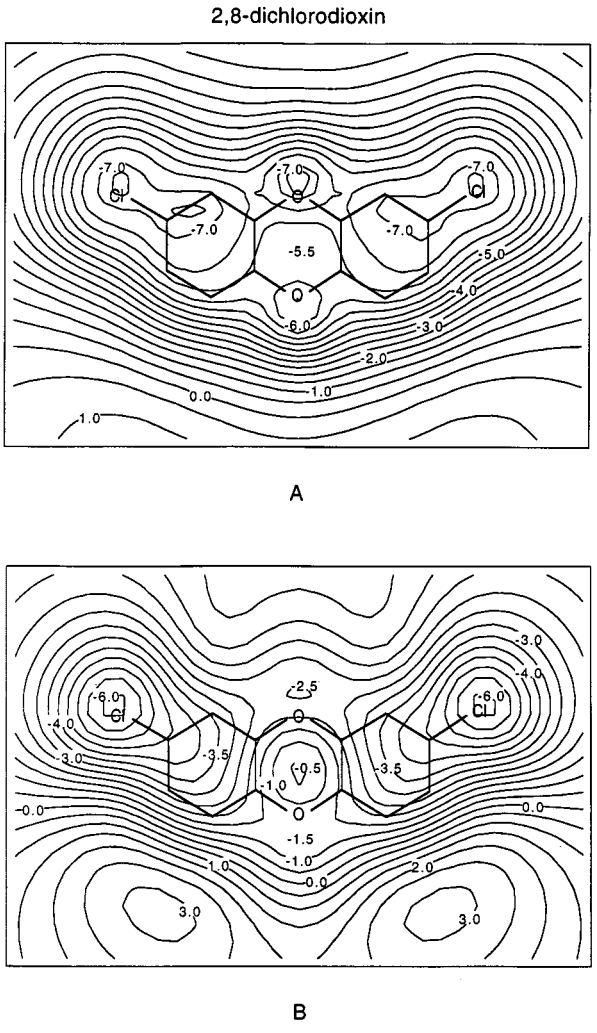

FIGURE 6. Same as Figure 2 except there are chlorine atoms substituted in the 2 and 8 positions of the dioxin.

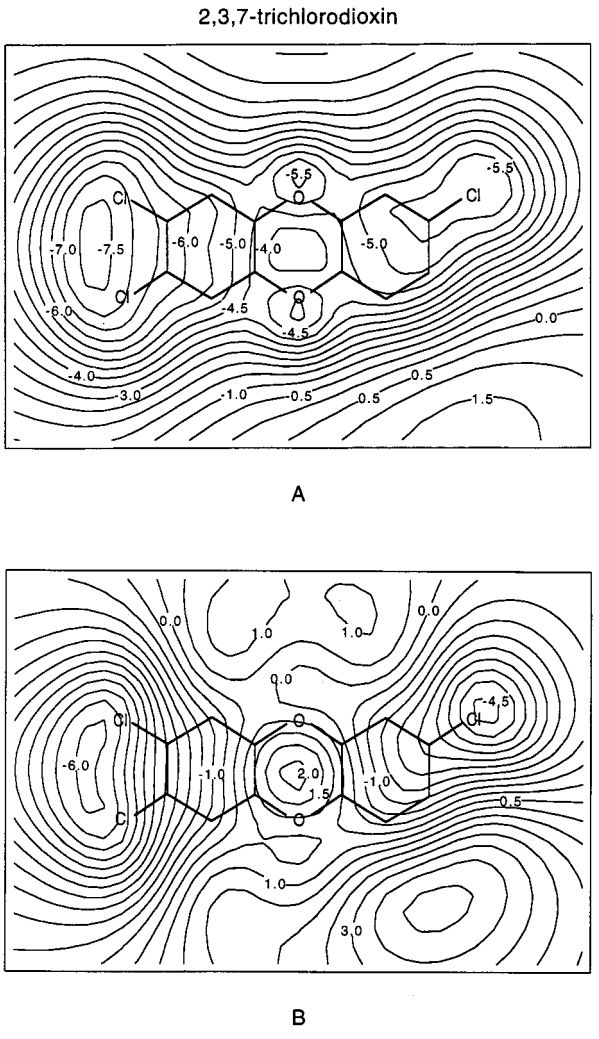

FIGURE 7. Same as Figure 2 except there are chlorine atoms substituted in the 2, 3, and 7 positions of the dioxin.

atoms. With the addition of chlorine atoms to the dioxin structure, all differences between the PIP and the MEP increase.

A similar electrostatic interaction potential was calculated using an atomic point charge model for both the dioxin molecule and the probe (not shown). The charges used in the interaction were the potential derived charges (CHELPG)[11] obtained from *ab initio* wave functions and the probe was moved in a plane parallel to the plane of the dioxin molecule. This is similar to the procedure that would be used for determining the electrostatic part of the interaction in standard classical potential methods for ligand–receptor interactions. Again, this interaction potential is always less stable than the PIP. The greatest difference, 6.5 kcal/mol, is above the conjugated ring just off its center toward the oxygen atoms. The magnitude of this difference varies little with chlorination of the dioxin but decreases monotonically as the probe is moved away from the conjugated rings in a plane parallel to the dioxin. The secondary maximum outside the lateral chlorine or hydrogen atoms disappears.

The movement of the positively charged probe in a plane parallel to the dioxin molecule effects the charge distribution of the dioxin molecule. This change in charge may be monitored by computing the charges on various atoms or groups of atoms in the dioxin molecule. The sum of the atomic charges on the C2X (either H or Cl) pair of atoms is most negative when the probe is over that bond and least negative when the probe is near the

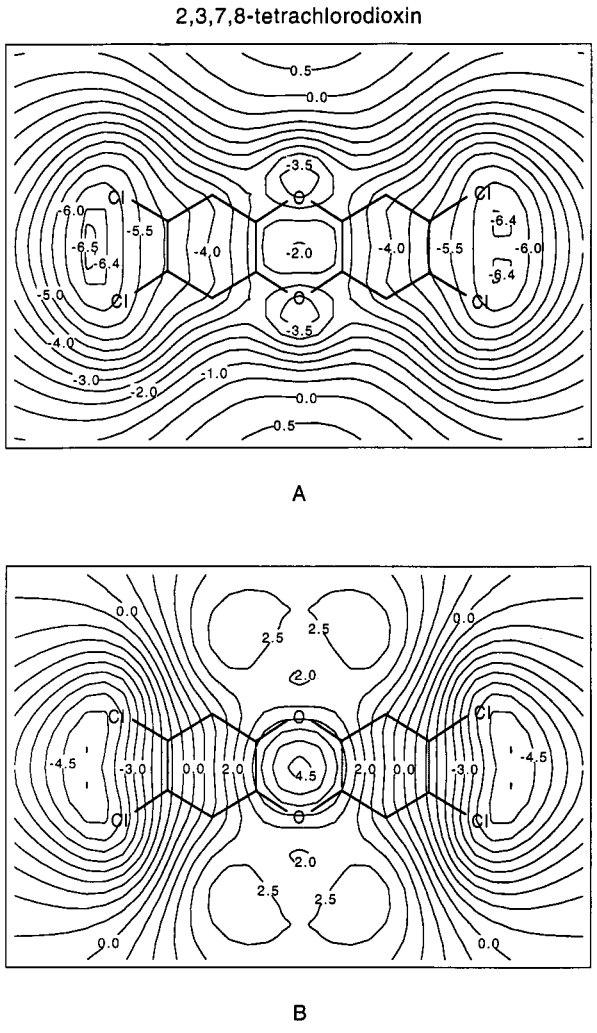

FIGURE 8. Same as Figure 2 except there are chlorine atoms substituted in the 2, 3, 7, and 8 positions of the dioxin (TCDD).

diagonal oxygen (O5). The difference is 0.1 electrons. As the probe moves further away from the C2X the charge slowly becomes more negative. For C2H the sign of the charge changes as a function of the position of the probe, whereas for C2Cl that group charge is always negative. The sum of the charges on each of the conjugated rings also varies by 0.1 electrons as a function of the position of the probe. The charge on the oxygen atoms varies by an order of magnitude less as a function of the position of the probe. Although the actual values of the group charges depend on the number of chlorine atoms in the molecule (and the group) the change in group charge due to the probe position does not depend significantly on dioxin chlorination.

The probe also causes a change in the molecular charge distribution perpendicular to the plane of the molecule. The polarization of the molecular charge distribution perpendicular to the molecular plane can be observed from the dipole moment of the molecule. Table I gives the dipole moment of the dioxin molecule for specified positions of the probe. The dipole moment of the ligand has been computed, using the Mulliken approximation,[15] to separate the dioxin dipole from the probe dipole (see ref. 13). The dipole moment is nearly independent of the origin chosen because the ligand remains essentially uncharged. The component of the dipole moment perpendicular to the molecular plane (d_y) is greatest when the probe is centered over the conjugated ring. It increases as the number of chlorine atoms on the ring increases. The components of the dipole moment in the plane of molecule are also influenced by the position of the probe. The changes in these components are a result of the movement of the charge between atoms and the polarization of atomic charge. Because these are single point calculations, and the dioxins are planar molecules, the component of the molecular dipole moment perpendicular to the plane of the molecule can result only from local polarization of the charge distribution and two center charge distributions shared between atoms in the dioxin and atoms probe. The latter contribution is small (0.1 d to 0.3 d). To obtain a similar-sized dipole moment perpendicular to the plane of the ligand, an electric field of 0.03 electrons/Å is required.

To further understand the interaction between the triMA cation and the dioxin molecules and the relevance of the PIP, the most stable bimolecular structures have been computed using the local minima in the PIP as starting points. There are four types of local minimum energy structures obtained (Fig. 9). The relative stabilization for each minimum is shown in Table II. The individual phenyl rings remain planar. However, the interaction with the probe causes the molecule to bend about the O—O axis. When the probe is near the chlorine atom(s) there is a local minimum with a small bend. If it is between two chlorine atoms (D) the bend is 1°. If it is above a single chlorine (C) the dioxin bend is 5°. For the local minimum energy structure, where the probe is above the π-system of a conjugated ring (A), the bend in the dioxin molecule is 15°. The most stable structure is when the probe is above an oxygen atom (B) and the triMA cation forms a hydrogen bond with a dioxin oxygen. This interaction causes a 30° bend

TABLE I.
Dipole Moments for Dibenzo-*p*-Dioxins under the Influence of Probe at Position Indicated (Debye).

	Δd_x	Δd_y	Δd_z
Dibenzo-*p*-dioxin			
No probe	0.00	0.00	0.00
Over ring[a]	−0.16	−0.79	−1.47
Over O	−0.69	−0.74	−0.28
Over H	−0.47	−0.43	−1.99
H—H[b]	−0.09	−0.42	−2.12
2-Chloro-dibenzo-*p*-dioxin			
No probe	−1.03	0.00	−2.13
Over ring[a]	−0.76	−0.83	−1.11
Over ring[c]	−0.78	−0.87	−3.49
Over O	−1.63	−0.79	−2.25
Over Cl	−1.40	−0.64	−4.15
Cl—H[b]	−0.93	−0.56	−4.30
2,3-Dichloro-dibenzo-*p*-dioxin			
No probe	0.00	0.00	−3.68
Over ring[a]	0.16	−0.84	−2.44
Over ring[d]	−0.13	−0.97	−4.85
Over O	−0.74	−0.82	−3.55
Over Cl	−0.46	−0.73	−5.67
Cl—Cl[b]	−0.11	−0.73	−5.94
2,7-Dichloro-dibenzo-*p*-dioxin			
No probe	0.00	0.00	0.00
Over ring[e]	−0.15	−0.90	−1.43
Over O	−0.73	−0.81	−0.30
Over Cl	−0.49	−0.68	−2.16
Cl—H[b]	0.05	−0.60	−2.33
2,8-Dichloro-dibenzo-*p*-dioxin			
No probe	−2.04	0.00	0.00
Over ring[e]	−1.67	−0.90	−1.44
Over O	−2.52	−0.82	−0.26
Over Cl	−2.38	−0.68	−2.16
Cl—H[b]	−1.84	−0.60	−2.34
2,3,7-Trichloro-dibenzo-*p*-dioxin			
No probe	−1.01	0.00	−1.56
Over ring[c]	−0.75	−0.91	−0.38
Over ring[d]	−0.77	−0.99	−2.77
Over O	−1.64	−0.85	−1.56
Over Cl	−1.45	−0.77	−3.66
Cl—Cl[b]	−0.82	−0.77	−3.95
2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin			
No probe	0.00	0.00	0.00
Over ring[e]	−0.12	−1.00	−1.43
Over O	−0.77	−0.88	−0.25
Over Cl	−0.53	−0.78	−2.27
Cl—Cl[b]	−0.10	−0.78	−2.55

[a]The ring with no lateral Cl atoms.
[b]The midpoint between lateral atoms indicated.
[c]The ring with one lateral Cl atom.
[d]The ring with two lateral Cl atoms.
[e]The two rings are equivalent relative to Δd_y.

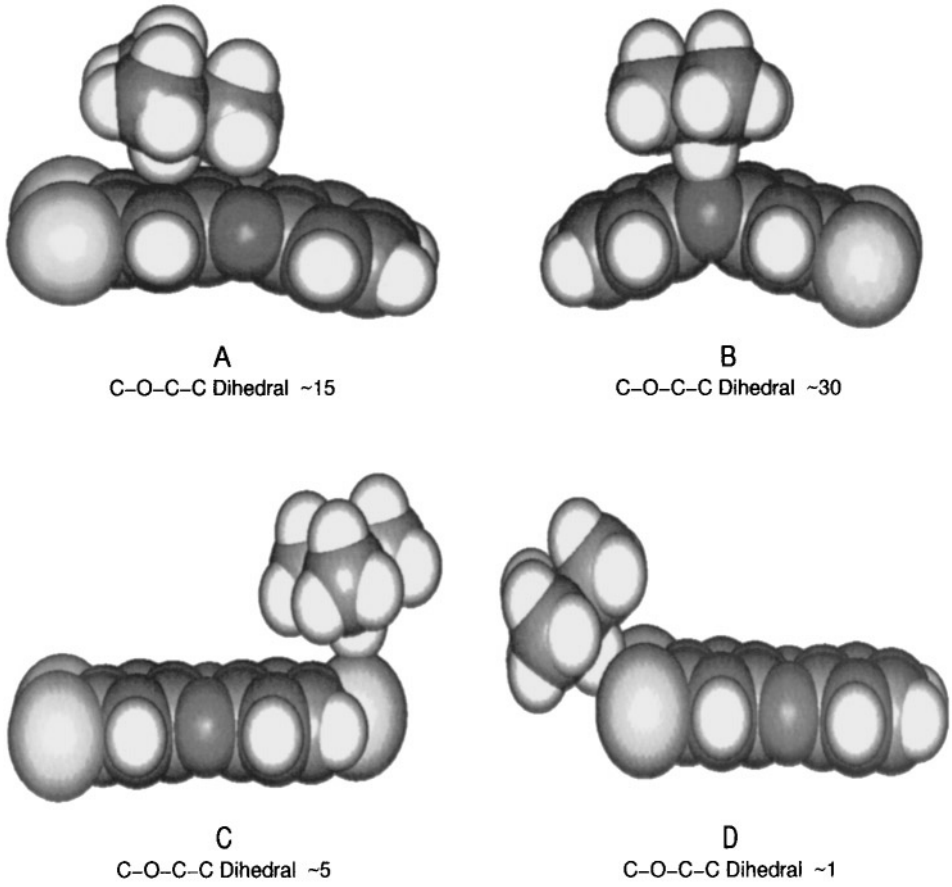

FIGURE 9. Four types of stable structures obtained for the interaction of dioxins with the trimethylammonium probe. The C—O—C—C dihedral bend of the dioxin is indicated.

in the dioxin. The orientation of the probe relative to the dioxin plane varies in the most stable bimolecular structures. For minimum energy structures A and C the probe is tipped so that C—H bonds from the methyl groups point toward the oxygen atoms in the dioxin. For type D structures the N—H bond forms a 47° angle with the dioxin plane. For the most stable structures, B, the probe tips slightly so that a C—H bond from a methyl group points in the direction of an oxygen atom. Although there is a one-to-one correspondence between the local minimum in the PIP and the stable structures, the order of these minima is not the same. The computed energies of the complex structure favor the complexes where the probe is above the oxygen, whereas, in the most stable PIP minima, the probe is between two chlorine atoms. For highly chlorinated dioxin molecules the MEP fails to indicate any minimum over the oxygen.

TABLE II.
Relative Energies of Minimized Structures (kcal / mol).

	A	B	C	D
Dioxin	6.84	0.00		
2-Cl		0.00	8.15	
23-Cl	6.68	0.00		4.30
27-Cl	6.19	0.00	6.86	
28-Cl	6.28	0.00	6.92	
237-Cl		0.00	6.00	3.05
2378-Cl		0.00		1.94

Discussion

The interaction between the (chlorinated) dioxin ligand, and a positively charged nitrogen-centered probe in a plane parallel to the ligand plane and 3.5 Å above, is always attractive. As the number of chlorine atoms in the ligand increases, the magnitude of similar minima in the PIP of each molecule decreases, but all local minima remain. For the

MEP, however, the local minimum over the oxygen atoms disappears and that region becomes repulsive as the number of chlorine atoms in the ligand increases. This represents the largest difference between the two potentials. This agrees with previous studies[16–18] of dioxin MEPs where it was shown that the electrostatic potential in the plane of dioxin had local minima near the oxygen atoms and that these minima disappeared as the number of lateral chlorine substitutions increased. This difference between the PIP and the MEP may be understood by considering the effect the chlorine atoms have on the charge distribution of the ring. The addition of chlorine atoms to the dioxin does not change the atomic charge on the oxygen, calculated using the Mulliken definition of charge (but does affect the potential derived charges). As chlorines are added to the lateral carbon atoms of the dioxin, the conjugated rings are polarized in the plane of the molecule. The MEP is computed from and reflects this charge distribution. The positive region of the MEP over the central ring of the highly chlorinated dioxins reflects the polarization of the adjacent conjugated rings. The induced dipole moment of each conjugated ring has its positive ends near the central ring. The contribution of this dipole overcomes the contribution to the MEP of the negatively charged oxygen atoms in highly chlorinated molecules. In addition, the nuclear charge of the dioxin ligand is in the plane of the ring, while the electronic charge extends from the plane. The addition of lateral chlorine atoms reduces the charge in the π-system which extends farthest from the molecular plane. Although the atom positions are fixed, the PIP includes the polarization of the electronic charge distribution of the ligand due to the presence of the probe (and the polarization of the probe due to the ligand). The polarization terms increase the attractive interaction between the probe and the ligand. The charge distribution of the ligand under the influence of the positively charged probe is polarized both in the plane of the molecule and perpendicular to that plane. The in-plane component is reflected in the partitioning of the charge between the two conjugated rings, the local atomic charges, and the dipole moment of each ring. This component reverses some of the ring polarization caused by the lateral chlorine atoms. The in-plane polarization of the charge distribution is not necessarily reflected in the molecular dipole moment of the ligand when the probe is over the center of the system because local contributions to the molecular dipole moment cancel, but these same local components add in the interaction energy. The out-of-plane component of the polarization of the molecular charge distribution is reflected in d_y of the molecule or the sp_y components of the electronic charge distribution. The out-of-plane molecular dipole moment is reflective of the strength of the interaction between the probe and the induced local moments perpendicular to the plane of the ligand because each of these local moments points in the same direction. Each component clearly depends on the position of the probe. In this study, we have not decomposed the ligand charge distribution into atomic or segmental multipole moments[13,19] to determine quantitatively the effect of each type of moment on the interaction energy. In a study in progress we plan to perform this decomposition for a number of conjugated systems.

In addition to the change in ligand charge distribution induced by the probe, the finite extent of the probe is another potential source of difference between the MEP and the PIP. To understand the effect of the latter, a similar potential for each of the dioxins in this study was computed using a point charge description of the probe (not shown). This potential is always very similar to the MEP. The maximum difference between the MEP and the PIP just beyond the lateral atoms is not seen when comparing this potential to the PIP. The difference between the MEP and the PIP above the central ring remains, but with this potential the maximum difference is nearer the C—C bonds in the central ring. This suggests that the differences between the potentials above the central ring of the ligand are a result of the redistribution of the electronic charge of the ligand under the influence of the probe.

The general features of the MEP and PIP are similar, with the exception of the discrepancy above the central ring of highly chlorinated ligands. In that region, a major feature of the interaction is lost by the MEP. For both potentials the most stable minimum is between two chlorine atoms if they are on the same side of the ligands. For each potential, as chlorine atoms are added to the ligand, the local minima become less stable. For most regions of the plane, the electrostatic term in the interaction is the largest; however, the interaction due to the polarization of the charge distribution also makes a large, differential contribution. This is in agreement with a similar interaction between benzene and NH_4^+.[9]

Both the PIP and the MEP yield multiple minima and therefore indicate multiple potential binding sites for positively charged species. Only the

TABLE III.
N—H—O Geometry for B Structures (Ranges for all Seven Structures).

N—H	1.03 Å
O—H	(1.75–1.80) Å
N—H—O	(166.5–168.7°)

PIP retains the local minima associated with the oxygen for highly chlorinated dioxin molecules. When the three-dimensional structure of the complex is allowed to relax, the most stable structure is obtained from the PIP minimum above the oxygen (B in Fig. 9). If only the MEP were considered for starting points, this lowest energy structure may not have been located. For this structure there is the largest deviation from planarity. The molecule bends by 30° about an axis defined by the two oxygen atoms. A hydrogen bond between the NH of the probe and the oxygen is formed. The N—H—O geometry is shown in Table III. The bend in the ligand increases the distance between the positive region of the dipole of each conjugated ring and the positive charge of the probe. This type of bend by dioxin molecules requires little energy as each conjugated ring remains planar. A flapping motion about this axis has been described in a study of the low-frequency spectra of dioxin.[20] A theoretical study[21] showed that a bend of 30° about this axis requires 0.8 kcal/mol, which is more than compensated for by the electrostatics and the putative hydrogen bond.

Conclusion

The cation probe interaction potential and the molecular electrostatic potential for the dioxins have many common features. They both show local minima associated with the π-system of the conjugated ring and the chlorine atoms of chlorinated dioxins. The major differences are: (1) in a plane parallel to the dioxin molecule the PIP retains local minima above the oxygen atoms in highly chlorinated molecules, whereas the MEP does not; and (2) the PIP is always attractive near the dioxin molecule, whereas the MEP has regions that are repulsive. The difference between the PIP and the MEP results primarily from the perturbation of the ligand charge distribution by the probe. This perturbation results in a component of the dipole moment perpendicular to the plane of the dioxin molecule that increases as the number of chlorine atoms increases: it is 1.0 debye for the dioxin with four lateral chlorine substitutions.

This series of molecules shows the influence of the π-cation interaction. There is a local minimum associated with the π-system of each of the conjugated rings. In the highly chlorinated molecules in this series that minimum is secondary to the minima associated with the chlorines. The π-cation interaction is largest when there is little substitution on the ring.

The multiple minima in the PIP were used as starting points for computing stable structures of the complex. The most stable structures for each molecule were obtained from local minima associated with the oxygen atoms. In such structures, a hydrogen bond was formed between the probe and the oxygen, and the molecule was bent by 30° about the O—O axis. In this series of molecules, the PIP retains some of the features that determine the hydrogen bond, whereas the MEP does not.

Acknowledgments

The authors thank Drs. A. Richard, J. McKinney, and L. Birnbaum for discussions relating to the research in this study. The computations in this study were performed at the National Environmental Supercomputer Center of the U.S. Environmental Protection Agency. The research has been reviewed by the National Health and Environmental Effects Research Laboratory of the U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policy of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

1. W. Bourguet, M. Ruff, P. Chambon, H. Gronemeyer, and D. Moras, *Nature*, **375,** 377 (1995).
2. J.-P. Renaud, N. Rochel, M. Ruff, V. Vivat, P. Chambon, H. Groneneyer, and D. Moras, *Nature*, **378,** 681 (1995).
3. J.-M. Wurtz, W. Bouguet, J.-P. Renaud, V. Vivat, P. Chambon, D. Moras, and H. Gronemeyer, *Nature Struct. Biol.*, **3,** 87 (1996).
4. C. L. Waller, T. I. Oprea, K. Chae, H.-K. Park, K. S. Korach, S. C. Laws, T. E. Weise, W. R. Kelce, and L. E. Gray Jr., *Chem. Rev. Toxicol.*, **8,** 1240 (1996).
5. K. S. Korach, P. Sarver, K. Chae, J. A. McLachlan, and J. D. McKinney, *Mol. Pharmacol.*, **33,** 120 (1988).
6. S. K. Burley and G. A. Petsko, *FEBS Lett.*, **203,** 139 (1986).

7. D. A. Dougherty, *Science*, **271,** 163 (1996).
8. R. A. Kumpf and D. A. Dougherty, *Science*, **261,** 1708 (1993).
9. H. Basch and W. J. Stevens, *Theochem*, **338,** 303 (1995).
10. M. J. Frisch, G. W. Trucks, M. Head-Gordon, P. M. W. Gill, M. W. Wong, J. B. Foresman, B. G. Johnson, H. B. Schlegel, M. A. Robb, E. S. Repogle, R. Gomperts, J. L. Andres, K. Raghavachari, J. S. Binkley, C. Gonzalez, R. L. Martin, D. J. Fox, D. J. Defrees, J. Baker, J. J. P. Stewart, and J. A. Pople, *Gaussian-92* (revision C), Gaussian, Inc., Pittsburgh, PA, 1992.
11. C. M. Breneman and K. B. Wiberg, *J. Comput. Chem.*, **11,** 361 (1990).
12. S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta Jr., and P. Weiner, *J. Am. Chem. Soc.*, **106,** 765 (1984).
13. J. R. Rabinowitz and S. B. Little, *Int. J. Quantum Chem.*, **13,** 9 (1986).
14. M. N. Ringnalda, J.-M. Langlois, R. B. Murphy, B. H. Greeley, C. Cortis, T. V. Russo, B. Marten, R. E. Donnelly Jr., W. T. Pollard, Y. Cao, R. P. Muller, D. T. Mainz, J. R. Wright, G. H. Miller, W. A. Goddard III, and R. A. Friesner, *PS-GVB* (version 2.3), Schrodinger, Inc., 1996.
15. R. S. Mulliken, *J. Chem. Phys.*, **23,** 1833 (1955).
16. J. S. Murray, B. A. Zilles, K. Jayasuriya, and P. Politzer, *J. Am. Chem. Soc.*, **108,** 915 (1986).
17. P. Politzer, *Toxicol. Lett.*, **43,** 257 (1988).
18. J. S. Murray, M. E. Grice, P. Politzer, and J. R. Rabinowitz, *J. Comput. Chem.*, **11,** 112 (1990).
19. J. R. Rabinowitz, T. J. Swissler, and R. Rein, *Int. J. Quantum Chem.*, **S6,** 353 (1972).
20. J. Grainger, V. V. Reddy, and D. G. Patterson Jr., *Chemosphere*, **18,** 981 (1989).
21. T. Fujii, K. Tanaka, H. Tokiwa, and Y. Soma, *J. Phys. Chem.*, **100,** 4810 (1996).